Reduction in biological efficacy of ethoprophos in a soil from Greece due to enhanced biodegradation: comparing bioassay with laboratory incubation data

Dimitrios G Karpouzas,[1]* Ioannis O Giannakou,[2] Allan Walker[1] and Simon R Gowen[2]

[1]*Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, UK*

[2]*University of Reading, Department of Agriculture, Earley Gate, TOB1, Reading, RG6 6AT, UK*

Abstract: Soils were collected from a potato-growing area in Serres, Northern Greece, where the nematicide ethoprophos was reported to have lost its effectiveness against cyst nematodes following 30 years of regular use. Incubation studies with ethoprophos and two bioassays using root-knot nematodes demonstrated that, in this heavily treated soil, the nematicide was degraded rapidly and nematicidal activity persisted only up to 14 days. In soil from an adjacent field with no known history of nematicide use during the preceding 14 years, ethoprophos was degraded more slowly and retained its nematicidal activity for more than 35 days. Ethoprophos efficacy was extended when the soil that had been treated in the field was autoclaved, although the effect was only transitory. The addition of 'pre-conditioned' soil from the previously treated field to samples of soil from the previously untreated field resulted in a significant acceleration of ethoprophos degradation compared with that observed in unamended soil from the previously untreated field.

Keywords: organophosphates; chemical control; enhanced microbial degradation; root-knot nematode; *Meloidogyne javanica*

1 INTRODUCTION

Since their introduction in the 1940s, nematicides have provided an important means of controlling nematodes.[1] They are classified according to their movement in soil following application as either fumigants or non-fumigants.[2,3] Ethoprophos (*O*-ethyl *S,S*-dipropyl phosphorodithioate) is a non-fumigant organophosphorus nematicide used in potato cultivation in Greece for the control of the potato cyst nematodes *Globodera rostochiensis* (Woll) and *G pallida* Stone.[4] It is also recommended for the control of *Meloidogyne* spp in tomato crops and for the control of *Ditylenchus dipsaci* (Kühn) Filipjer in onions and strawberries. It is used as a pre-planting, granular application at a dose of 7–10 kg AI ha^{-1} which is incorporated into the first 10–12 cm of the surface soil. The applied ethoprophos should persist for 6–8 weeks at a concentration sufficient to provide satisfactory protection of a potato crop.

Recently, rapid degradation of ethoprophos has been reported in soils from previously treated potato fields in Greece,[5] where the nematicide concentration was degraded to undetectable levels in about two weeks. This contrasts with its persistence in a similar soil from a previously untreated field in which 30% of the initial dose was still present six weeks after application.[5] Similar rapid degradation following repeated application has been reported for granular nematicides such as ethoprophos, aldicarb and oxamyl in the Netherlands[6] and the United States.[7] These rapid rates of loss were assumed to be due to enhanced biodegradation by a fraction of the soil microflora that had adapted to transform the chemicals.[8]

In the present study, both incubation and bioassay techniques were used to determine if the rapid degradation of ethoprophos observed in incubation studies was associated with loss of efficacy in bioassay experiments. An experiment was also made to determine whether the potential for rapid biodegradation of ethoprophos could be transferred from one soil to another.

2 EXPERIMENTAL METHODS

2.1 Pesticide and soils

Analytical grade ethoprophos (99%; Promochem,

* Correspondence to: Dimitrios G Karpouzas, Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, UK
E-mail: dimitrios.karpouzas@hri.ac.uk

Contract/grant sponsor: State Scholarship Foundation of Greece.
(*Received 23 February 1999; revised version received 10 June 1999; accepted 6 August 1999*)

UK) was used as the reference compound in analytical operations as well as in the incubation and bioassay studies. Soil samples were collected on 27 December 1997 from a potato monoculture area in Northern Greece. Approximately 35 kg of surface soil (10–12 cm) was collected from a field with a 30-year history of annual application of ethoprophos (10G Mocap, Rhone-Poulenc, Greece). Samples were collected from two points separated by 20–25 m from an area of the field where the owner had noticed high nematode infestation on potato roots. Samples of soil with no history of ethoprophos treatment were also collected from a nearby site on 4 January 1998. This second field had been fallow for at least 10 years up to 1996, since when it has been used for pesticide-free 'organic' production of potatoes. The soil samples were stored at 4 °C until their transfer to the UK. On arrival, the soils were partially air-dried overnight, sieved to pass a 3-mm mesh, and their residual moisture content was determined by oven drying sub-samples at 110 °C for 24 h. Maximum water-holding capacities of the soils were measured gravimetrically following saturation of the soil (25 g) with distilled water in a filter funnel with Whatman No 1 filter paper and allowing to drain for 24 h. Water content at 40% water-holding capacity gives a first approximation of soil moisture content at a soil water potential of −33 kPa.[9]

2.2 Chemical determination of ethoprophos persistence

Duplicate samples of 400 g soil from the previously treated and the previously untreated field were spread separately on polyethylene sheet, and drops of a solution of ethoprophos (4 ml; 500 mg litre^{-1} in methanol) added over the surface of each to give a dose of 5 mg kg^{-1}. This dose approximates to that recommended for the control of potato cyst nematodes. Soils were left for four to five hours for the solvent to evaporate before sufficient distilled water was added to adjust soil moisture contents to 40% of the water-holding capacity. Samples were thoroughly mixed by hand, transferred to pre-sterilized polyethylene bottles (500 ml; Merck, UK) and incubated at 15 °C for eight weeks. The degradation rate of ethoprophos was determined by removing 20-g samples 0, 7, 13, 18, 21, 28, 35, 42, 48 and 56 days after the initial pesticide addition. Ethoprophos residues were extracted with methanol (25 ml) and analysed by GLC as described in previous studies.[5] Recoveries of ethoprophos from soil ranged between 90 and 98% and the limit of detection was approximately 0.005 mg kg^{-1}. The moisture content of the incubated samples was maintained at 40% of their water-holding capacity by adding distilled water, when necessary, at each sampling date. It was assumed that losses of ethoprophos resulted primarily from degradation in the soil, and that volatility losses from the incorporated treatment and closed containers would be relatively insignificant.

2.3 Transfer of degrading ability from a previously treated soil to a previously untreated soil

On the assumption that enhanced biodegradation of ethoprophos would have occurred in soil from the previously treated field, a sub-sample (500 g) of this soil was treated with ethoprophos as described above on four occasions at intervals of seven days. This procedure was expected to maximize the rapid ethoprophos-degrading potential of this soil.[5] A sub-sample (2 kg) of soil from the previously untreated field was divided into 10 amounts of 200 g. Fractions of 0.2, 1, 2 and 10 g of soil were removed from eight of these sub-samples and the amounts removed were replaced by the same amounts of the 'pre-conditioned' rapid-biodegradation soil. This procedure produced duplicate 200-g amounts of the previously untreated soil containing 0, 0.1, 0.5, 1 and 5% of the 'pre-conditioned' soil. Ethoprophos in methanol (2 ml; 500 mg litre^{-1}) was added dropwise to each sample to give a pesticide concentration of 5 mg kg^{-1} dry soil. Further duplicate samples of 200 g from the previously treated soil and from the 'preconditioned' soil were treated in exactly the same way. After the addition of ethoprophos, all samples were handled as before (Section 2.2), and distilled water was added to bring the moisture content of the soil to 40% of its water-holding capacity. Subsequently, the soil samples were transferred to sterile polypropylene bottles and incubated at 15 °C for 42 days. Immediately after application of the pesticide and 6, 10, 14, 18, 21, 24, 28, 31 and 42 days later, sub-samples (20 g) were removed from the soil and analysed for ethoprophos residues. The moisture content of the incubated soil samples was maintained constant throughout the experiment by addition of distilled water at each sampling date.

2.4 Bioassay determination of ethoprophos efficacy

To monitor the changes in ethoprophos efficacy, four 5-kg samples of soil from the previously treated field and two 5-kg samples of soil from the previously untreated field were weighed into polyethylene bags. Two of the soil samples from the previously treated field were autoclaved twice (successive days) at 123 °C for 20 min. One replicate sample of the autoclaved soil, the non-autoclaved soil from the previously treated field, and one sample from the previously untreated field received an application of ethoprophos by addition of 25 ml of a 1000 mg litre^{-1} ethoprophos solution in methanol to give a dose of 5 mg kg^{-1}. The second replicate of the three soils received a similar amount of methanol without ethoprophos. All soil samples were handled in different areas of the laboratory (*c* 5 m apart) in order to minimize the possibility of cross-contamination between the different treatments. Soils were left for 4–5 h for the solvent to evaporate and the appropriate amount of distilled water was added to bring the moisture content to 50% of the maximum water-holding capacity of the soil.

This moisture content was chosen to be conducive to nematode survival and invasion of the plant roots. Soils were mixed by hand and then passed three times through sterilized 3-mm mesh sieves to ensure uniform distribution of the chemical. Subsequently, all of the six bulk soil samples were individually divided into 35 lots of 100 g and 28 lots of 50 g which were transferred to sterilized polypropylene bottles and incubated at 15 °C for 42 days. The moisture content was maintained by regular additions of distilled water. Immediately after treatment and 7, 14, 21, 28, 35 and 42 days later, the efficacy of the residual ethoprophos was assessed by bioassay in two experiments using *Meloidogyne javanica* (Treub) Chitwood. Root-knot nematodes (*Meloidogyne* spp) have a life cycle that is similar to that of cyst nematodes. In addition, both nematodes have the same infective stage, which is the second stage juvenile (J2). They were used in our experiment because they are easier to culture and mass produce for experimental purposes than cyst nematodes.

2.4.1. Experiment 1: Direct counting of extracted nematodes from soil

Four samples of 50 g from each treatment were removed from the incubator at each date, placed in beakers (100 ml) and inoculated with 1000 freshly hatched second-stage juvenile nematodes (J2s). Beakers were covered with aluminium foil to prevent water evaporation and returned to the 15 °C incubator. After 24 h, the inoculated soils were placed on small extracting trays with water and kept at room temperature for three days. The water from the trays was subsequently collected into beakers and left for 3 h for the nematodes to settle. The top layer of the water from each beaker was removed until 30 ml remained. The numbers of J2s were then counted in three 1-ml aliquots of the residual solution.

2.4.2. Experiment 2: Counting of female nematodes extracted from infected roots

At each sampling date, five samples of 100 g soil from each treatment were removed from the incubator. Each sample was placed in a separate beaker, inoculated with nematodes as described above and incubated at 15 °C for 24 h. After 24 h, a tomato seedling (*Lycopersicon esculentum* (Mill) cv 'Tiny Tim'; four leaf stage) was transplanted into the soil in each beaker. The plants were placed in the greenhouse (25–30 °C) and 10 days later, all tomato plants with the soil ball were transplanted into 250-cm^3 plastic pots containing a loam-based compost (John Innes No 2). Thirty-five days later, the plants were uprooted and the stem removed. The roots were washed free of soil and females on roots counted using a stereoscopic microscope with 12.5 × magnification.

The data obtained from the bioassay experiments were analysed separately (ANOVA) using GENSTAT[10] following transformation of the data to $\log_{10}(n+1)$, where n refers to the number of nematodes.

3 RESULTS

3.1 Ethoprophos degradation in laboratory-based incubations

The degradation pattern of ethoprophos in the laboratory incubation is shown in Fig 1. Variability between replicates was less than 5%, and mean values are presented in Fig 1 for the two soils. Less than 7% of the initial dose remained in the soil from the previously treated field after incubation for 13 days, and degradation of ethoprophos in this soil was complete within 21 days of treatment. In contrast, in soil from the previously untreated field, more than 60% of the added ethoprophos was still present 13 days after pesticide application, and about 11% of the applied dose was still detected in the soil after 56 days.

3.2 Soil mixing experiment

The results (Fig 2) once more confirmed the rapid degradation of ethoprophos in soil from the previously treated field compared with the slow degradation in the soil from the previously untreated field. Variability between the two replicates of each treatment was less than 5%, and mean values are presented in Fig 2 for each treatment. Very rapid dissipation of ethoprophos was observed in the 'preconditioned' samples from the previously treated field, where ethoprophos residues disappeared within eight days. In soil samples from the previously treated field and in samples from the previously untreated field mixed with 5% of the 'preconditioned' soil, degradation of ethoprophos was complete in 18 and 21 days, respectively (Fig 2). Residual amounts of 13.5, 3.6 and 2.9% of the initial concentration, were detected 21 days after addition of the pesticide to samples from the previously untreated field mixed with 0.1, 0.5 and 1% of the 'preconditioned' soil, respectively. At the same time, more than 43% of the initial dose of ethoprophos was detected in the unamended samples from the previously untreated field. Degradation of ethoprophos was complete in 31 days in samples of soil from the previously untreated field amended with 0.1% 'preconditioned' soil,

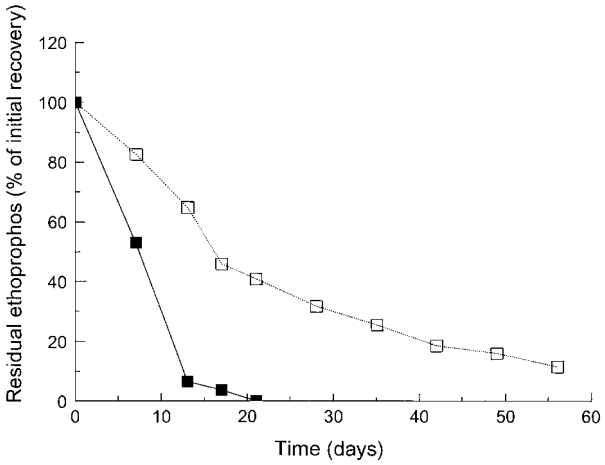

Figure 1. Degradation of ethoprophos in soil from (■) a previously treated and (□) a previously untreated field, in the laboratory. Each value is the mean of two replicates.

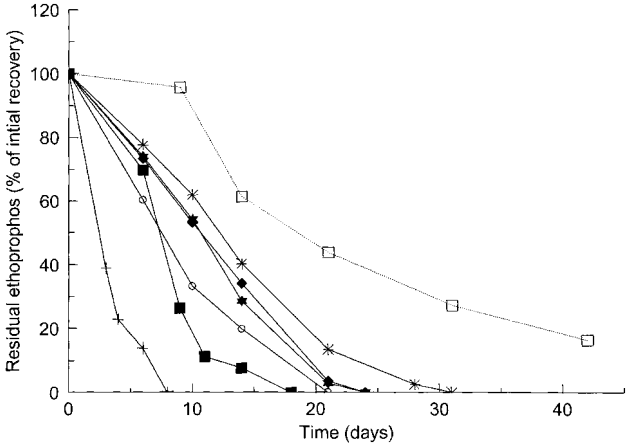

Figure 2. Degradation of ethoprophos in (+) 'preconditioned' soil from the previously treated field, unamended soils from (■) the previously treated and (□) the previously untreated fields, or soil from the previously untreated field mixed with (✳) 0.1 %, (◆) 0.5 %, (★) 1% and (○) 5% of the 'preconditioned' soil. Each value is the mean of two replicates.

whereas in unamended soil from this site, about 20% of the initial amount of ethoprophos was still present 42 days after addition of the nematicide.

3.3 Bioassay determination of ethoprophos degradation

There was no significant difference ($P = 0.05$) in the number of J2s extracted from the soil from the previously treated field which received 5 mg kg^{-1} of ethoprophos and the corresponding untreated control 21 days after the pesticide addition (Fig 3a). In contrast, there were always significantly fewer J2s extracted from the samples treated with ethoprophos (5 mg kg^{-1}) than from the untreated controls (0 mg kg^{-1}) when soil from the previously untreated field (Fig 3b), and sterilized soil from the previously treated field were examined (Fig 3c). There was no significant difference ($P = 0.05$) in the numbers of females in tomato roots between soil from the previously treated field which received ethoprophos and the corresponding utreated control, seven days after the nematicide application (Fig 4a). Significantly fewer ($P < 0.05$) *M javanica* females parasitized tomato roots in the soil from the previously untreated field to which a new addition of ethoprophos had been made than in the respective control samples, at all sampling dates (Fig 4b). As in the extraction experiment, there was a gradual increase in the number of females colonizing the roots in the sterilized soil from the previously treated field (Fig 4c). However, significantly fewer ($P < 0.05$) females were counted at all sampling times up to 28 days, in the tomato roots in the sterilized soil from the previously treated field with the new addition of ethoprophos, compared with the controls where no ethoprophos was added.

4 DISCUSSION

Enhanced degradation of ethoprophos in soils from potato fields with a history of extensive ethoprophos

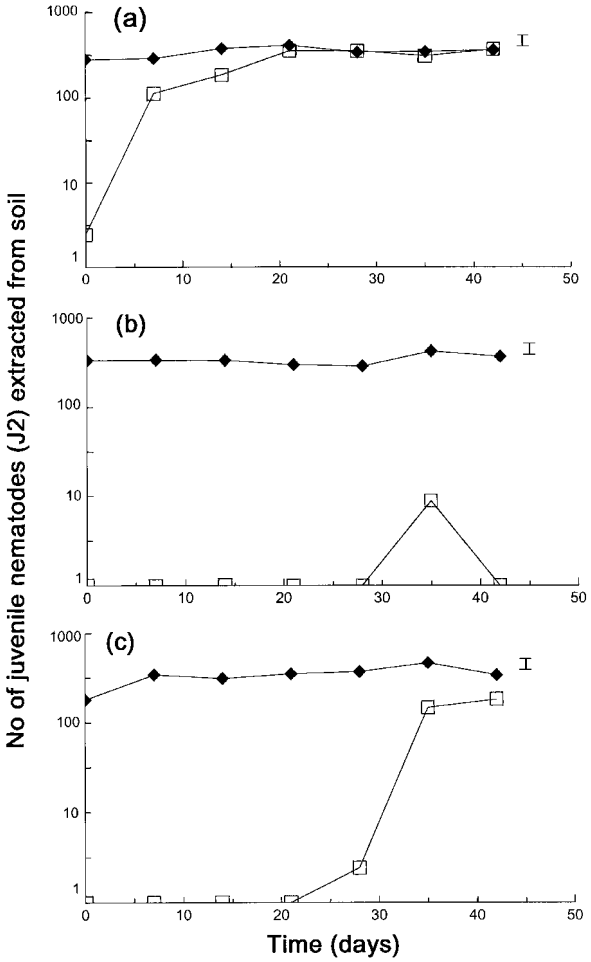

Figure 3. Numbers of *Meloidogyne javanica* juveniles extracted from soils taken from fields (a) previously treated with ethoprophos for 30 years (b) previously untreated with ethoprophos and (c) previously treated but sterilized in the laboratory; (□) samples with a fresh addition of 5 mg kg^{-1} ethoprophos, (◆) control samples (0 mg kg^{-1}). Each value is the mean of four replicates. The vertical bar represents the least significant difference ($P = 0.05$) for comparing data points derived by ANOVA following transformation of the data to $\log_{10}(n+1)$

use was confirmed using incubation and bioassay studies. Previous exposure to ethoprophos enhanced the degradation rate of a further ethoprophos addition, compared with that in a soil never exposed previously to the nematicide. There was good agreement between the results from the incubation studies and those from the bioassays, which suggests that laboratory incubation studies can be used as an indicator of potential field efficacy. However, the stability and efficacy of a pesticide treatment in the field will depend upon the extent to which the potential degradative capacity of a soil is expressed under field conditions.[11]

The properties of the two soils used are listed in Table 1. There were small differences in pH, and in extractable nitrate-nitrogen and potassium, but it seems unlikely that these can explain the differences in the observed degradation rates of ethoprophos between the soils. However, organic matter content and extractable phosphorus levels in the soil from the previously treated field were much greater than those in the soil from the previously untreated field. Organic

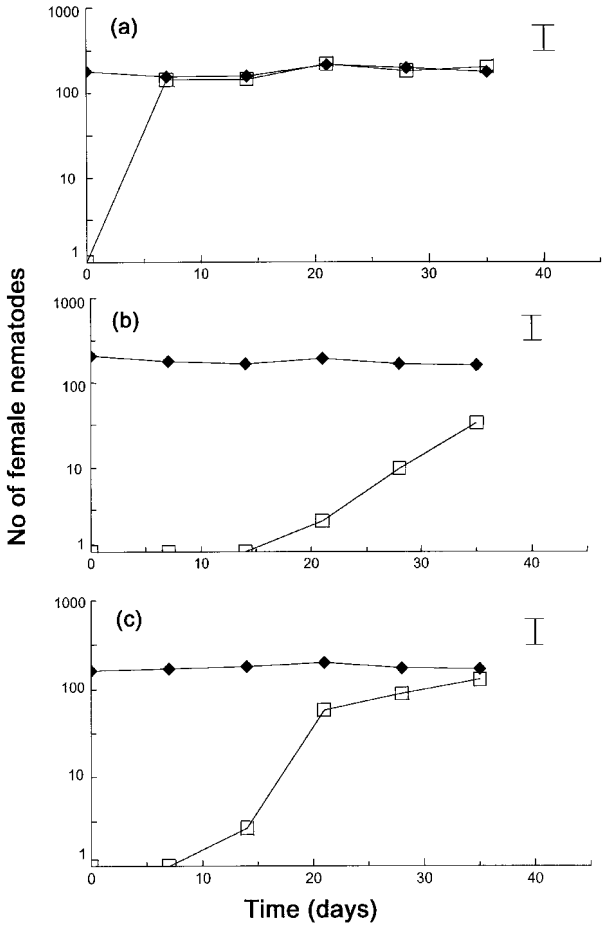

Figure 4. Numbers of *Meloidogyne javanica* females in tomato roots after inoculation with 1000 juveniles in soils taken from a field (a) previously treated with ethoprophos for 30 years (b) previously-untreated with ethoprophos and (c) previously treated but sterilized in the laboratory; (□) samples with a fresh addition of 5 mg kg^{-1} ethoprophos; (◆) control samples (0 mg kg^{-1}). Each value is the mean of five replicates. The vertical bar represents the least significant difference ($P = 0.05$) for comparing data points derived by ANOVA following transformation of the data to $\log_{10}(n+1)$

matter content can play a significant role in determining pesticide behaviour in soils through the broad positive correlation of both soil microbial activity and pesticide sorption with soil organic matter content.[12] Jones and Norris,[13] after reviewing previous studies of ethoprophos degradation in soil, concluded that its persistence generally increases with increased levels of soil organic matter, and suggested that the dominant factor was reduced bioavailability of the pesticide at increased soil organic matter levels. Previous studies in soils from the University of Athens, Greece, showed considerably faster degradation of ethoprophos in samples where the nematicide had been applied nine months previously than in soil from a previously untreated field at the same site. The two soils examined had very similar pH values and organic matter contents, although nutrient status was not assessed.[5] It was concluded that microbial adaptation in response to applications of ethoprophos can be a major factor leading to differences in degradation rate between soils, as had been suggested previously in similar studies in the Netherlands.[8] In addition, complete disappearance of ethoprophos in five days was reported in a field study where the soil had been treated annually with the nematicide for the preceding three years.[14] The soil used in the present experiments had received an annual application of ethoprophos for the previous 30 years, and this appears to have resulted in the development of a microbial population with the ability to degrade the nematicide rapidly enough to produce a significant reduction in its biological efficacy. The phenomenon of accelerated biodegradation has been reported previously with other organophosphorus nematicides and insecticides such as fenamiphos[15,16] isofenphos,[17,18] fonofos,[19] phorate and chlorfenvinphos.[20]

The results from the soil-mixing experiment confirm that the differences in ethoprophos degradation rate were related to microbiological differences and not to physicochemical differences between the two soils. There was a progressive increase in ethoprophos degradation rate as the proportion of 'preconditioned' soil from the previously treated field in the mixtures was increased (Fig 2). Amounts of 'preconditioned' soil as small as 0.1% were enough to increase the degradation rate of ethoprophos in the mixed samples compared with that in the unamended soil from the previously untreated field. This further reinforces the suggestion that microbiological processes are the main determinant of rapid degradation, since it is unlikely that incorporation of such small amounts of soil from the previously treated field could alter the physicochemical properties of the soil from the previously untreated field. The data in Fig 2 are consistent with those reported in similar studies with the dicarboxamide fungicides iprodione and vinclozolin; incorporation of small amounts of a 'preconditioned' soil into a soil in which fungicide degradation was relatively slow dramatically increased the rates of the fungicides loss.[21]

The accelerated degradation of ethoprophos observed in the incubation studies was also supported by the results from the bioassays using *M javanica* as a biomonitor organism. The rapid reduction in ethoprophos persistence in the soil from the previously

Table 1. Characteristics of soils used in the study

Soil	pH	Organic matter (%)	Moisture content at 40 % WHC (% w/w)	N	P	K
				(mg $litre^{-1}$)		
Previously-treated	6.1	2.72	21.0	11	54	279
Previously untreated	5.5	1.39	16.7	15	22	209

treated field resulted in a dramatic decrease in nematode mortality. Because a period of 30–40 days is needed for the hatching of nematodes in the field, the nematicide must persist for at least this period in order to be effective. Our data demonstrate that ethoprophos residues adequate to provide sufficient control of nematodes persisted for less than 20 days in the soil from the previously treated field. The gradual increase in the numbers of surviving J2s and females observed in the sterilized soil from the previously treated field at the later stages of the study was not unexpected. There are difficulties in handling such large amounts of soil under sterile conditions, and in maintaining sterility over extended time periods. Recolonization of the autoclaved soil by micro-organisms that either survived autoclaving or were re-introduced from the surrounding environment would be possible. Such bacteria would readily proliferate in an autoclaved soil where there would be little competition for energy sources.

The reduction in efficacy of ethoprophos in commercial potato fields in Greece has potential to develop into a practical economic problem since ethoprophos costs significantly less than the alternative nematicides fenamiphos, aldicarb or oxamyl. Rotation of ethoprophos use with that of other nematicides is one possible alternative, although previous studies in the Netherlands have shown that, once established, enhanced degradation of ethoprophos can last for three to five years.[6] However, studies examining the stability of the enhanced degradation of ethoprophos with time under the specific local soil and weather conditions are essential to determine the correct management strategies in Greece. Additional experiments are also required to investigate the occurrence of cross-enhancement between ethoprophos and other nematicides (cadusafos, fenamiphos, aldicarb, oxamyl, fonofos).

ACKNOWLEDGEMENTS

Dimitrios Karpouzas and Ioannis Giannakou would like to thank the State Scholarship Foundation of Greece for financial support. Special thanks are given to Mr Troubatas and the owners of the field in Northern Greece for their help. We are grateful to Mrs K Phelps, HRI, Wellesbourne, for statistical analysis of the bioassay data.

REFERENCES

1 Johnson AW, The role of nematicides in nematode management, in *Advanced Treatise on Meloidogyne*. Vol 1, *Biology and Control*, ed by Sasser JN and Carter CC, North Carolina State University Graphics. pp 249–267 (1985).
2 Spurr HW, Mode of action of nematicides, in *Advanced Treatise on Meloidogyne*. Vol 1, *Biology and Control*, ed by Sasser JN and Carter CC, North Carolina State University Graphics. pp 268–297 (1985).
3 Hague NGM and Gowen SR, Chemical control of nematodes, in *Principles and Practice of Nematode Control in Crops*, ed by Brown RH and Kerry BR, Australia Academic Press. pp 133–178 (1987).
4 Whitehead AG, *Plant nematode control*. CAB International, Wallingford (1998).
5 Karpouzas DG, Walker A, Froud-Williams RJ and Drennan DSH, Evidence for the enhanced degradation of ethoprophos and carbofuran in soils from Greece and the stability of the phenomenon in previously treated soils. *Pestic Sci* **55**:301–311 (1999).
6 Smelt JH, Van de Peppel - Groen AE, Van de Pas LJT and Dijkesterhuis A, Development and duration of accelerated degradation of nematicides in different soils. *Soil Biol Biochem* **28**:1757–1765 (1996).
7 Mojthedi H, Santo GS and Pinkerton JN, Efficacy of ethoprop on *Meloidogyne hapla* and *M chitwoodi* and enhanced biodegradation in soil. *J Nematol* **23**:372–379 (1991).
8 Smelt JH, Crum SJH, Teunissen W and Leistra M, Accelerated transformation of aldicarb, oxamyl and ethoprophos after repeated soil treatments. *Crop Protection* **6**:295–303 (1987).
9 Cox L and Walker A, Studies of time-dependent sorption of linuron and isoproturon in soils. *Chemosphere* **38**:2707–2718 (1998).
10 Genstat 5 Committee, *Gentstat TM 5 Release 3 Reference Manual*, Oxford, Clarendon Press (1993).
11 Suett DL and Jukes AA, Stability of accelerated degradation of soil-applied insecticides: laboratory behaviour of aldicarb and carbofuran in relation to their efficacy against cabbage root fly (*Delia radicum*) in previously treated soils. *Crop Protection* **12**:431–442 (1993).
12 Hurle K and Walker A, Persistence and its prediction, in *Interactions Between Herbicides and the Soil*, ed by Hance RJ, London Academic Press. pp 83–122 (1980).
13 Jones RJ and Norris FR, Factors affecting degradation of aldicarb and ethoprop. *J Nematol* **30**:45–55 (1998).
14 Rhode WA, Johnson AW, Dowler CG and Glaze NC, Influence of climate and cropping patterns on the efficacy of ethoprop, methyl bromide and DD-MENCS for control of root-knot nematodes. *J Nematol* **12**:33–39 (1980).
15 Stirling AM, Stirling GR and Macrae IC, Microbial degradation of fenamiphos after repeated application to a tomato-growing soil. *Nematologica* **38**:245–254 (1992).
16 Davis RF, Johnson AW and Wauchope RD, Accelerated degradation of fenamiphos and its metabolites in soil previously treated with fenamiphos. *J Nematol* **25**:679–685 (1993).
17 Chapman RA, Harris CR, Moy P and Henning K, Biodegradation of pesticides in soil: rapid degradation of isofenphos in a clay loam after a previous treatment. *J Environ Sci Health B* **21**:269–276 (1986).
18 Racke KD and Coats JR, Enhanced degradation of isofenphos by soil microorganisms. *J Agric Food Chem* **35**:94–99 (1987).
19 Wilde G, Enhanced degradation of fonofos in soil from two southwest Kansas fields. *J Econ Entomol* **83**:1250–1253 (1990).
20 Suett DL and Jukes AA, Evidence and implications of accelerated degradation of organophosphorus insecticides in soil. *Toxic Environ Chem* **18**:37–49 (1988).
21 Walker A and Welch SJ, Enhanced biodegradation of dicarboxamide fungicides in soil, in *Enhanced Biodegradation of Pesticides in the Environment*, ed by Racke KD and Coats JR, American Chemical Society Symposium Series. pp 53–67 (1990).